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Dysregulation of tyrosine kinases and use of imatinib in small animal practice

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ABSTRACT

Imatinib inhibits the activity of several tyrosine kinases, including BCR-ABL, KIT and platelet-derived growth factor receptor (PDGFR). Dysregulation of KIT is found in mast cell tumours (MCTs) and *KIT* is mutated in approximately 30% and 70% of canine and feline MCTs, respectively. *KIT* mutations have also been reported in canine and feline gastrointestinal stromal tumours (GISTs), canine acute myeloid leukaemia and canine melanoma. In addition, *BCR-ABL* and *PDGFR* mutations have been found in canine leukaemia and haemangiosarcoma, respectively. Imatinib has anti-tumour activity with tolerable toxicity towards a certain subset of MCTs in dogs and cats. Favourable clinical responses are likely to be associated with the presence of *KIT* mutation. Anti-tumour activity of imatinib has also been demonstrated in canine GISTs with a *KIT* mutation and in feline hypereosinophilic syndrome; however, to date only one of each of these cases has been reported. In conclusion, analysis of *KIT* mutations appears to provide valuable data for individual treatment with imatinib in dogs and cats.

Introduction

Since the discovery of KIT dysregulation due to mutations in canine mast cell tumours (MCTs) (London et al., 1999; Ma et al., 1999), veterinary oncology research with a focus on targeted therapy using tyrosine kinase inhibitors (TKIs) has dramatically increased. Two TKIs (toceranib and masitinib) have entered the clinic (Hahn et al., 2008; London et al., 2009) and are currently changing the therapeutic approach for malignancies in dogs and cats.

The small-molecule TKI, imatinib (Novartis; also known as Gleevec or Glivec), is a prototype of target-oriented drugs in humans. It has sparked a revolution in cancer therapy by dramatically improving treatment for chronic myeloid leukaemia (CML) in humans (Druker et al., 2001). Imatinib targets BCR-ABL, KIT, platelet-derived growth factor receptor (PDGFR), colony stimulating factor 1 receptor, ABL1, ABL2, discoidin domain receptor 1/2 and lymphocyte-specific protein tyrosine kinase (Deininger et al., 2005; Dewar et al., 2005; Manley et al., 2010).

Some tumours in dogs and cats possess mutations in these tyrosine kinases; therefore, imatinib is one of a number of potential target-oriented therapeutic approaches for canine and feline neoplasms. The aim of this article is to review the current knowledge regarding the dysregulation of kinases and the therapeutic effects of imatinib in canine and feline neoplasms, particularly in MCTs.

KIT and other potential targets for imatinib in canine and feline tumours

Among the potential targets for imatinib, KIT, which is expressed in MCTs, has been the most extensively studied in dogs and cats. To date, there is only limited information available regarding other potential targets in canine and feline tumours.

KIT mutations in canine mast cell tumours

The reported location and frequency of *KIT* mutations in canine MCTs are summarised in Table 1. *KIT* mutations have been observed in approximately 30% of canine MCTs (Letard et al., 2008; Takeuchi et al., 2013). *KIT* mutations are most frequently found in exon 11 (~14–21%) and primarily consist of internal tandem duplication (ITD) mutations (9–17%) in randomly selected MCT cases (Zemke et al., 2002; Webster et al., 2006; Letard et al., 2008; Takeuchi et al., 2013). Other than exon 11 mutations, mutations have been found in exons 2, 6, 7, 8, 9, 15 and 17 (Letard et al., 2008; Takeuchi et al., 2013). Compared with mutations in exon 11, mutations in exons 8 and 9 are less frequent, although a significant number of mutations have been identified (Letard et al., 2008; Takeuchi et al., 2013). Mutations in other exons are generally infrequent (<3%).

A high incidence of exon 11 ITD mutations has been reported in higher grade MCTs classified according to Patnaik grades (Downing et al., 2002; Zemke et al., 2002; Webster et al., 2006). Although no significant differences in the frequency of the exon 11 ITD mutation were observed in MCTs across Patnaik grades, the frequency

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Table 1
Reported frequency of *KIT* mutation in randomly selected canine mast cell tumours.

Number of cases	Region of *KIT* examined	Frequency of mutation (%)					Reference
		Exon 8	Exon 9	Exon 11 (ITD)	Other exons	Total	
47	Entire *KIT*	6.4	0	21.3 (17.0)	<3	34.0	Takeuchi et al. (2013)
191	Exons 8–13 and 17–19	4.7	4.2	16.8 (13.1)	<3	26.2[a]	Letard et al. (2008)
88	Exon 11	NA	NA	13.6 (9.1)	NA	NA	Zemke et al. (2002)
60	Exon 11	NA	NA	NA (13.3)	NA	NA	Webster et al. (2006)

NA, not available; ITD, internal tandem duplication mutation.
[a] Value indicates the total frequency of mutations in exons 8–13 and 17–19.

of this mutation in Kiupel high grade MCTs was significantly higher than that in low grade tumours (Takeuchi et al., 2013). Moreover, the presence of exon 11 ITD mutation is associated with an increased incidence of recurrent disease, metastasis, death or shorter progression-free survival (Downing et al., 2002; Webster et al., 2006; Takeuchi et al., 2013). All these reports suggest an association between clinical aggressiveness and the presence of ITD mutations, suggesting that imatinib may have therapeutic utility for canine MCTs with aggressive behaviour.

Although mutated KIT is an important target for imatinib in canine MCTs, genetic heterogeneity within the same tumour and/or between different tumours from the same patient (primary tumour vs. metastasis) could exist. In humans, the presence of intratumoral genetic heterogeneity influences therapeutic response and contributes to resistance against kinase inhibitors (Bedard et al., 2013). However, in dogs, Marconato et al. (2014) observed no difference in the mutation status of *KIT* between primary canine MCTs and their corresponding metastases. In contrast, Amagai et al. (2013) reported that two cases of canine MCT had an ITD mutation in the primary lesion but not in the secondary lesion. The latter finding implies the presence of heterogeneity in *KIT* mutational status among different geographical regions of MCTs, similar to that reported for various human tumours (Bedard et al., 2013). Genetic heterogeneity could therefore be an issue when using imatinib therapy for canine MCTs.

KIT mutation in feline mast cell tumours

The mutational status of *KIT* in feline MCT is summarised in Table 2. Isotani et al. (2010) found that 67.7% of feline MCTs had a mutation in *KIT*. The majority of the mutations were identified in exon 8, where they mostly consisted of an ITD mutation, and in exon 9. Sabattini et al. (2013) reported that 62.5% of cats with MCT had a mutation in at least one of their multiple nodules and that the majority of these mutations were found in exons 8 and 9. Although the most frequent mutation was reported to be in exon 8 (45.2% of cases) in the study of Isotani et al. (2010), it was reported to be in exon 9 (50.0% of cases) in the study of Sabattini et al. (2013). The reason for this difference is unclear, but could reflect a difference in the tumour population (e.g. visceral vs. cutaneous). Although Dank et al. (2002) investigated *KIT* mutation in exons 11, 12 and 17 in 10 cats with MCT, no mutation was identified. Thus, *KIT* mutations appear to be more frequent in feline MCTs than canine MCTs, and the 'hotspot' of mutation differs from that of canine MCTs. Furthermore, in contrast to canine MCTs, no significant relationship between *KIT* mutation status and tumour behaviour was observed for feline MCT (Sabattini et al., 2013).

Sabattini et al. (2013) demonstrated that multiple nodules from the same cat had different *KIT* mutational status; one cat had two tumour nodules that each showed a different mutation, while five cats had tumour nodules in the same subject with or without *KIT* mutations. As speculated for canine MCTs, some feline MCTs may have genetic heterogeneity in the same animal.

KIT mutations in canine and feline gastrointestinal stromal tumours

KIT mutations have been detected in canine and feline gastrointestinal stromal tumours (GISTs). In dogs, Frost et al. (2003) reported that 2/4 cases had a deletion/insertion or a substitution mutation (*KIT* exon 11 was examined) and Gregory-Bryson et al. (2010) reported that 6/17 cases had deletion mutations in *KIT* exon 11 (*KIT* exons 8, 9, 11, 13 and 17, and *PDGFRA* exons 12, 14 and 18, were examined). A deletion mutation in *KIT* exon 11 was also identified in a case of canine GIST (Kobayashi et al., 2013) and a deletion mutation in *KIT* exon 11 has been reported in a case of feline GIST (Morini et al., 2011). There was an overlap between canine and feline GIST mutations with one of the hotspots of driver mutations in human GIST (Corless et al., 2011). A substitution mutation reported by Frost et al. (2003) in canine GIST has also been reported in some human GISTs (Lasota et al., 1999). Although the number of cases examined is still small, these studies indicate that a certain subset of canine and possibly feline GISTs may share similar molecular features with human GISTs and that mutant KIT could be a potential therapeutic target of imatinib in these tumours.

KIT mutations in canine leukaemia and lymphoma

Usher et al. (2009) examined the mutation status of *KIT* exons 8, 10, 11 and 17 in canine acute leukaemias (myeloid, lymphoid and undifferentiated). In this study, 3/21 cases of acute myeloid leukaemia had *KIT* mutations (one mutation in each of exon 11 and 17, one mutation in exon 17 or two mutations in exon 17). In contrast, no *KIT* mutation was found in cases of acute lymphoid leukaemia ($n = 14$) or acute undifferentiated leukaemia ($n = 1$). Giantin et al. (2013a) also investigated the presence of mutations

Table 2
Reported frequency of *KIT* mutation in randomly selected feline mast cell tumours.

Number of cases	Region of *KIT* examined	Frequency of mutation (%)						Reference
		Exon 6	Exon 8 (ITD)	Exon 9	Exon 11	Other exons	Total	
62	Entire *KIT* or exons 8, 9, 11, 13, and 17	5.9[a]	45.2 (40.3)	24.2	1.6	0	67.7	Isotani et al. (2010)
24	Exons 8, 9, and 11	NA	16.7[b] (8.3[b])	50.0[b]	8.3[b]	NA	62.5[b]	Sabattini et al. (2013)

NA, not available; ITD, internal tandem duplication mutation.
[a] Mutation in exon 6 was examined in 17 cats and one cat had a mutation.
[b] The frequency of mutation was recalculated from the original report as the percentage of cats that had a mutation in at least one of their multiple nodules.

Table 3
Canine and feline tumours expressing KIT or platelet-derived growth factor receptor.

	Tumour[a]	Species	Detection	Reference
KIT	Mast cell tumour	Dog	IHC	London et al. (1996), Gil da Costa et al. (2007)
		Cat	IHC	Rodriguez-Cariño et al. (2009), Sabattini and Bettini (2010)
	Gastrointestinal stromal tumour	Dog	IHC	Frost et al. (2003), Morini et al. (2004)
		Cat	IHC	Morini et al. (2004), Morini et al. (2011)
	Melanoma	Dog	IHC	Murakami et al. (2011), Newman et al. (2012), Chu et al. (2013)
	Leukaemia/lymphoma	Dog	RT-PCR, FACS, IHC	Giantin et al. (2013a), Giantin et al. (2013b)
	Mammary gland carcinoma	Dog	RT-PCR, IHC	Kubo et al. (1998), Morini et al. (2004), Brunetti et al. (2014)
		Cat	IHC	Morini et al. (2004)
	Anal sac adenocarcinoma	Dog	RT-PCR, IHC	Brown et al. (2012), Urie et al. (2012)
	Thyroid carcinoma	Dog	RT-PCR, IHC	Urie et al. (2012)
	Haemangiosarcoma	Dog	IHC	Sabattini and Bettini (2009)
	Seminoma	Dog	IHC	Morini et al. (2004), Hara et al. (2014)
	Interstitial cell tumour	Dog	IHC	Morini et al. (2004)
	Granulosa cell tumour	Dog	IHC	Morini et al. (2004)
		Cat	IHC	Morini et al. (2004)
	Ovarian papillary carcinoma	Dog	IHC	Morini et al. (2004)
	Soft tissue fibrosarcomas	Cat	IHC	Smith et al. (2009)
	Histiocytic sarcoma[b]	Dog	RT-PCR, FACS	Zavodovskaya et al. (2006)
PDGFRA/B	Anal sac adenocarcinoma	Dog	RT-PCR, IHC	Brown et al. (2012), Urie et al. (2012)
	Lymphoma	Dog	RT-PCR, IHC	Aricò et al. (2014)
	Thyroid carcinoma	Dog	RT-PCR, IHC	Urie et al. (2012)
	Haemangiosarcoma	Dog	IHC	Asa et al. (2012)
	Osteosarcoma	Dog	IHC	Maniscalco et al. (2013)
	Gliomas	Dog	IHC	Higgins et al. (2010)
	Vaccine-associated sarcoma[b]	Cat	WB analysis	Katayama et al. (2004)

PDGFRA/B, platelet-derived growth factor receptor-alpha/beta; IHC, immunohistochemistry; RT-PCR, reverse transcription-polymerase chain reaction; FACS, flow cytometry; WB, Western blot.
[a] Tumours with low frequency of expression were also included.
[b] Cell lines.

in *KIT* exons 8–11 and exon 17 in 11 cases of acute leukaemias (lymphoid, $n = 4$; undifferentiated leukaemia, $n = 7$) and in 12 cases of chronic lymphocytic leukaemias, but found no mutations.

All three cases of acute myeloid leukaemia with *KIT* mutations had a mutation in the kinase domain that altered Asp815 (corresponding to human Asp816) to Val ($n = 2$) or Asn ($n = 1$) (Usher et al., 2009). KIT with Asp816 mutations, especially the Asp816Val mutation, has been shown to be highly resistant to imatinib in humans because this mutation causes a conformational change in KIT towards the active form (Ma et al., 2002; Foster et al., 2004). Thus, canine acute myeloid leukaemias carrying KIT Asp815 mutations may not respond to imatinib.

KIT mutations in lymphoma were investigated by Giantin et al. (2013b), who examined mutations in *KIT* exons 8–11 and 17 in B cell lymphomas ($n = 25$) and T cell lymphomas ($n = 21$), but no mutation was identified.

KIT mutations in canine melanomas

Chu et al. (2013) examined the mutational status of *KIT* exon 11 in canine malignant melanomas that had developed at different anatomical sites ($n = 49$) and found a missense mutation in 1/33 case of oral melanoma. Murakami et al. (2011) also examined the mutational status of *KIT* exon 11 in canine oral melanoma ($n = 17$), but no mutation was identified. In humans, Beadling et al. (2008) demonstrated that 15.6% of mucosal melanomas displayed *KIT* mutations, which commonly occurred in exon 11, and that imatinib was effective for patients with mucosal melanoma that carried a *KIT* mutation (Hodi et al., 2013). Thus, in contrast to humans, *KIT* mutations seem to be uncommon in canine mucosal melanomas. Although *KIT* mutations other than those in *KIT* exon 11 need to be examined, canine mucosal melanomas may not respond to imatinib, since imatinib did not have a growth inhibitory effect on nine canine malignant melanoma cell lines (Ito et al., 2013).

BCR-ABL in canine leukaemia

Chromosomal translocations between the *ABL* gene on chromosome 9 and the *BCR* gene on chromosome 26, as well as the presence of the BCR-ABL fusion protein, have been demonstrated in a canine CML by fluorescence in situ hybridisation (FISH) and Western blot analysis, respectively (Breen and Modiano, 2008). The *BCR-ABL* translocation has also been detected in three dogs with chronic monocytic leukaemia (Cruz Cardona et al., 2011; Culver et al., 2013; Pérez et al., 2013) and in one dog with acute myeloblastic leukaemia (Figueiredo et al., 2012) by FISH analysis. Although the nucleotide sequence of DNA fusion junctions for *BCR-ABL* translocations has not yet been analysed, these findings suggest the presence of BCR-ABL in a certain subset of leukaemia in dogs, which would make imatinib a potential therapeutic approach for these canine tumours, similar to humans.

PDGFR mutation in canine haemangiosarcoma

Only one study has demonstrated the presence of *PDGFR* mutations in canine haemangiosarcomas (Abou Asa et al., 2013). In that report, 2/27 cases exhibited missense mutations in exon 14 or 17 of *PDGFRB*. No similar *PDGFRB* mutations have been reported for *PDGFRB* in human neoplasms. Further studies are needed to clarify the significance of these mutations for haemangiosarcoma.

Wild-type tyrosine kinases or tyrosine kinases whose mutation status is unknown in canine and feline tumours

Aberrant signalling of tyrosine kinases could be caused not only by specific mutations, but also by overexpression, which could increase the response of cancer cells to growth factors or induce receptor dimerisation in the absence of an activating ligand (Zwick et al., 2002). In dogs and cats, the expression of wild-type

tyrosine kinases or of tyrosine kinases whose mutation status is unknown has been reported for several tumours. For example, KIT and PDGFR are expressed in various tumours in dogs and cats (Table 3). This could have important therapeutic implications; however, it is not clear whether this is sufficient for a positive response with imatinib. In a recent study of human melanomas (Hodi et al., 2013), a response to imatinib was observed in tumours with *KIT* mutations, but not in tumours with amplification of wild-type KIT. In canine haemangiosarcoma cell lines, imatinib inhibited phosphorylation of PDGFRB; however, the half maximal inhibitory concentrations of imatinib for growth of these cells were 50–65 μM (Dickerson et al., 2013), which are substantially higher than the therapeutic concentrations reported in humans (Cmax of a standard dose of 400 mg is approximately 3 μM; Peng et al., 2004). Further investigation is needed to understand the relationship between the expression of KIT, PDGFR and other kinases, and the therapeutic response to imatinib.

The use of imatinib for dogs and cats in a clinical setting[1]

Imatinib was originally developed as a therapeutic drug for human CMLs that targets BCR-ABL (Druker et al., 1996). Subsequently, clinical studies indicated a remarkably high therapeutic response to imatinib by human GISTs, which frequently have *KIT* mutations (Debiec-Rychter et al., 2004). Imatinib has also been demonstrated to have anti-tumour activity towards several other tumours with kinase mutations, such as GISTs with a *PDGFRA* mutation (Heinrich et al., 2003), hypereosinophilic syndrome (HES) with a *FIP1L1-PDGFRA* fusion mutation (Cools et al., 2003), myelodysplastic/myeloproliferative neoplasms with *PDGFR* gene rearrangements (Verstovsek, 2007), mastocytosis with *KIT* mutations (except for the KIT D816V mutation) (Pardanani, 2013) and melanoma (mucosal, acral and chronically sun-damaged) with *KIT* mutations (Hodi et al., 2013).

Imatinib is a drug developed for humans that has been used off-label for dogs and cats, similar to most chemotherapeutic drugs in veterinary medicine. Due to the prohibitive cost of imatinib, it has been difficult to use imatinib in dogs and cats. However, less expensive generic versions of imatinib became available after 2013.

Mast cell tumours

In dogs, a total of 28 cases of MCTs treated with imatinib have been reported; of these, 25 cases were treated with imatinib (10–12.7 mg/kg daily), with examination of *KIT* mutation status (examination of entire *KIT*, *KIT* exons 8, 9 and 11, or *KIT* exon 11 alone) (Isotani et al., 2008; Yamada et al., 2011; Kobayashi et al., 2012; Nakano et al., 2014). In addition to these 25 cases, we have treated 13 cases with imatinib (10–12 mg/kg daily) with examination of *KIT* mutation status (*KIT* exons 8, 9, and 11) (unpublished data). Of these 38 cases, 16 cases had a detectable mutation in *KIT* exon 8, 9 or 11. All of these 16 cases achieved an objective response (complete or partial response) to imatinib. Of the 22 cases without a detectable *KIT* mutation, only five cases showed an objective response to imatinib. Three dogs with aggressive MCTs and an unknown *KIT* mutation status were treated with imatinib (4.4 mg/kg daily) and all of these dogs achieved a complete response (Marconato et al., 2008).

In cats, 11 cases of MCTs treated with imatinib (10 mg/kg daily) have been reported and all were examined for *KIT* mutation (examination of entire *KIT* or *KIT* exons 8, 9, 11, 13 and 17) (Isotani et al., 2006, 2010). Nine had a mutation in *KIT* exons 8 or 9 and all, except one cat with stable disease, achieved an objective response (complete or partial response) to imatinib. Of the two cats without detectable *KIT* mutations, one responded. In a phase I clinical trial of imatinib in tumour-bearing cats (Lachowicz et al., 2005), one case of MCT with an unknown *KIT* mutation status showed no objective tumour response after imatinib at a dose of 15 mg/kg daily.

Based on these previous reports, imatinib can be viewed as efficacious in certain canine and feline MCTs, and clinical responses are likely to be associated with *KIT* mutations. However, aberrant kinase phosphorylation in canine and feline MCTs could not be solely attributable to a mutant KIT. In dogs, five cases that responded to imatinib had no mutation within the entire nucleotide sequence of *KIT* (Isotani et al., 2008). Likewise, no *KIT* mutation was identified in one cat with MCT that responded to imatinib therapy, even though the entire nucleotide sequence of *KIT* was examined (Isotani et al., 2010). Therefore, other mechanisms may underlie the anti-tumour effect of imatinib in these cases. It is possible that imatinib may inhibit other aberrantly regulated tyrosine kinases; for example, mutations in *PDGFRA* and *PDGFRB* were found in humans with imatinib-sensitive mastocytosis (Pardanani et al., 2003; Lahortiga et al., 2008). Thus, activating mutations in other tyrosine kinases could be driving the response to imatinib in these *KIT* mutation negative cases. Alternatively, the samples that were used for nucleotide sequencing analysis of *KIT* may not have been of sufficient quality for mutation analysis (e.g. the purity of the tumour cells). This is possible, since some analyses used fine needle aspiration samples that could potentially be contaminated with a large number of non-neoplastic cells (e.g. blood cells, inflammatory cells and mesenchymal cells).

Most studies have used dosage levels of 10 mg/kg daily or higher; however, Marconato et al. (2008) successfully treated three dogs with MCTs with a lower dose of imatinib (4.4 mg/kg daily). Their findings indicate the possibility that a lower dosage of imatinib could be suitable for the treatment of canine and feline MCTs. If so, treatment costs and the incidence of side effects would be greatly reduced.

In summary, although imatinib has been shown to have anti-tumour activity towards canine and feline MCTs, the reported studies only include a small number of animals. Therefore, large-scale clinical studies are needed to clarify the clinical utility of imatinib for these tumours.

Other neoplasms and non-neoplastic disorders

In dogs, imatinib (10 mg/kg daily) has also been used to treat a dog with an unresectable GIST with a *KIT* exon 11 mutation and a tumour response was noted (Kobayashi et al., 2013). Currently there is no known effective chemotherapeutic drug for canine GISTs. Further studies interrogating the therapeutic effects of imatinib in canine GISTs are needed to clarify whether this canine tumour type is a good candidate for imatinib therapy, similar to humans.

A case of feline HES was treated with imatinib at a daily dose of 9.6 mg/kg (Backlund et al., 2011). This cat showed clinical improvement, with normalisation of peripheral eosinophil counts after imatinib therapy. It is possible that this feline tumour might have expressed an aberrant kinase, such as the FIP1L1-PDGFRA fusion kinase, similar to human HES; however, this was not investigated in that study.

In a phase I clinical trial of imatinib in cats (Lachowicz et al., 2005), all four cats with vaccine-associated fibrosarcoma (VAFS) showed tumour stabilisation (<50% change in tumour volume) for an average of 2 months. It is not known if feline VAFS cells have a driver mutation in a kinase that is targeted by imatinib. However, PDGF/PDGFR signalling has been shown to play an important role in the growth of feline VAFS and imatinib can inhibit the PDGF-stimulated growth of VAFS cells in vitro and in vivo (Katayama et al., 2004). It is therefore possible that imatinib may have suppressed tumour growth by interfering with PDGFR signalling.

[1] Response to imatinib was expressed according to the Response Evaluation Criteria in Solid Tumours (RECIST) guidelines in dogs (Nguyen et al., 2013) or humans (http://www.recist.com/; accessed 26 November 2014).

Interestingly, in addition to its use in oncology, imatinib (3 mg/kg daily) has also been examined for treatment of pulmonary arterial hypertension (PAH) in dogs (six cases) (Arita et al., 2013). Imatinib was clinically effective for PAH caused by mitral regurgitation or chronic filariasis in all six dogs, presumably by targeting PDGFR, which is a factor in the molecular pathogenesis of PAH in humans (Rabinovitch, 2008). That study suggests the potential therapeutic use of imatinib for diseases other than cancer.

Toxicity

The toxic effects associated with imatinib treatment of dogs and cats are summarised in Table 4. Isotani et al. (2008) treated 21 dogs with MCTs with imatinib (10 mg/kg) and no clinical or haematological toxicity, except for one dog that experienced mild vomiting, was noted. Furthermore no notable toxicity that could be related to imatinib (10–12.7 mg/kg) was reported in three other studies that included a total of four dogs with MCTs (Yamada et al., 2011; Kobayashi et al., 2012; Nakano et al., 2014). Of the 32 dogs with MCTs that we treated with imatinib (10–12 mg/kg), neutropenia, vomiting or elevation of serum liver enzymes, blood urea nitrogen (BUN) and serum creatinine were observed in a small number of cases (unpublished data). All of these toxicities improved following reduction or transient withdrawal of imatinib. An increase in serum alanine aminotransferase (ALT) was also observed in a canine case of GIST treated with imatinib (10 mg/kg), but serum ALT decreased to normal levels when the dose of imatinib was reduced (Kobayashi et al., 2013).

Both Marconato et al. (2008) and Arita et al. (2013) used a range of low doses of imatinib (3–4.4 mg/kg) for the treatment of MCT or PAH; no haematological or non-haematological adverse effects were observed. In an experimental setting, progressive liver toxicity was observed in normal dogs treated with a high dose of imatinib (100 mg/kg), but not in dogs treated with a dose of 3 mg/kg (Druker and Lydon, 2000).

In a phase I clinical trial, imatinib (1–15 mg/kg) was administered to 11 tumour-bearing cats, including cats with VAFS, MCT or squamous cell carcinoma, and no unacceptable toxicity was noted (two cats showed mild lethargy or mild vomiting) (Lachowicz et al. (2005). In the studies of Isotani et al. (2006, 2010), a total of 11 cats with MCTs were treated with imatinib (10 mg/kg) and no notable toxicity was observed. In contrast, one cat with HES treated with imatinib (9.6 mg/kg) developed proteinuric nephropathy (Backlund et al., 2011). We have also observed proteinuria in a cat with MCT treated with imatinib (10–12 mg/kg) (unpublished data). This cat also showed mild vomiting with mild to moderate increase of ALT, BUN and creatinine.

Based on these findings, imatinib at an approximate dose of 10 mg/kg or less appears to be well tolerated in dogs and cats, with some possibility of bone marrow, hepatic, renal and gastrointestinal toxicity in dogs, and hepatic, renal and gastrointestinal toxicity in cats.

Towards individualised treatment

Since imatinib is considered to have therapeutic activity towards a certain subset of canine and feline tumours with dysregulated KIT, it would be beneficial to individualise imatinib treatment according to the mutational status of *KIT*.

The characteristics of the reported canine and feline *KIT* mutations, and the effects of imatinib in vitro and in clinical cases, are summarised in Table 5. Fifteen out of 36 reported *KIT* mutations have been demonstrated to cause constitutive activation of KIT in vitro, suggesting that they are activating mutations. The most frequent

Table 4
Toxicities associated with imatinib treatment of dogs and cats.

	Number of cases	Disease	Dose (mg/kg)[a]	Treatment period (week)	Toxicities (grade)[b]	*n*	Reference
Dogs	21	MCT	10	1–9	Vomiting (1)	1	Isotani et al. (2008)
	3	MCT	4.4	11–21	None		Marconato et al. (2008)
	2	MCT	10–12.7	13 and 45	None		Nakano et al. (2014)
	1	MCT	10	10	None		Yamada et al. (2011)
	1	MCT	10	6	None		Kobayashi et al. (2012)
	32	MCT	10–12	1–43	Vomiting (1)	1	Our laboratory, unpublished data
					Neutropenia (1)	2	
					Increase in ALT (3)	1[g]	
					Increase in AST (3)	1[g]	
					Increase in ALP (2)	1[g]	
					Increase in BUN (1)	1[g]	
					Increase in Cr (2)	1[g]	
	1	GIST	7[c]–10	>120[d]	Increase in ALT (2)	1	Kobayashi et al. (2013)
	6	PAH	3	4	None		Arita et al. (2013)
Cats	11	Various[e]	1–15	2–8	Lethargy (1)	1	Lachowicz et al. (2005)
					Vomiting (1)	1	
	10	MCT	10	2–32	None		Isotani et al. (2010)
	1	MCT	10	33[f]	None		Isotani et al. (2006)
	1	HES	9.6	8	Proteinuria	1	Backlund et al. (2011)
	1	MCT	10–12	26	Vomiting (1)	1[h]	Our laboratory, unpublished data
					Proteinuria	1[h]	
					Increase in ALT (3)	1[h]	
					Increase in BUN (1)	1[h]	
					Increase in Cr (1)	1[h]	

MCT, mast cell tumour; GIST, gastrointestinal stromal tumour; PAH, pulmonary arterial hypertension; HES, hyperoeosinophilic syndrome; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Cr, creatinine.

[a] Imatinib was given daily except for the reported case of Kobayashi et al. (2013).

[b] Grade of toxicities were expressed according to Veterinary Cooperative Oncology Group (2011).

[c] Imatinib was given every other day.

[d] The reported period was 20 weeks but the treatment was continued for >120 weeks.

[e] Includes vaccine-associated fibrosarcoma ($n = 4$), squamous cell carcinoma ($n = 4$) and mast cell tumour ($n = 1$).

[f] The reported period was 5 weeks but treatment was continued until 33 weeks.

[g] Toxic events were observed in the same dog.

[h] Toxic events were observed in the same cat.

Table 5
Characteristics of reported canine and feline KIT mutations and effects of imatinib in vitro and in clinical cases.

KIT mutation			Tumour	Frequency (%)	Activation status	In vitro effects of imatinib	Clinical response to imatinib (*n*)
Dogs	Exons 6–7	p.Asp312_Thr414delinsAla	MCT	2.1[a]	NA	NA	NA
	Exon 8	p.Glu417_Thr420dup{Glu417Gln}	MCT	4.2[b], 6.4[a]	Activated[b,c]	Suppression[c]	PR (1)[c]
		p.Gln430Arg	MCT	0.5[b]	NA	NA	NA
	Exon 9	p.Ser479Ile	MCT	2.6[b]	Activated[b]	NA	NA
		p.Asn508Ile	MCT	1.6[b]	Activated[b,d]	Suppression[d]	PR (1)[d]
	Exon 11	p.Gln555_Lys557del	MCT	NA	NA	NA	CR (1)[e]
		p.Gln555_Lys557delinsVal	MCT	0.5[b]	Activated[b]	NA	NA
		p.Trp556Arg	MCT	NA	Activated[f]	NA	NA
		p.Trp556_Lys557del	MCT	0.5[b]	Activated[f]	NA	NA
			GIST	11.2[g]			NA
		p.Trp556_Lys557delinsGln	GIST	25.0[h]	NA	NA	NA
		p.Trp556_Val558delinsPhe	GIST	23.5[g]	NA	NA	PR (1)[i]
		p.Lys557delInsAsnPro[v]	MCT	2.1[b]	NA	NA	NA
		p.Lys557_Val559delinsArg	MCT	0.5[b]	NA	NA	NA
		p.Val558del	MCT	2.1[a]	Activated[f]	NA	NA
		p.Val559Asp	MCT	NA	NA	NA	CR (1)[e]
		p.Leu575Pro	MCT	2.1[a]	Activated[f,j]	NA	NA
			GIST	25.0[h]			NA
			MEL	2.0[k]			NA
			AML	4.8[l]			NA
		Various ITD around codon 570–590	MCT	9.1–17.0[a,b,m,n]	Activated[b,f,j,o,p]	Suppression[p]	CR/PR (16)[q,r]
	Exon 15	p.Ser714del	MCT	2.1[a]	NA	NA	NA
	Exon 17	p.Asp815Asn	AML	4.8[l]	NA	NA	NA
		p.Asp815Val	AML	9.5[l]	NA	NA	NA
		p.Lys817Arg	AML	4.8[l]	NA	NA	NA
		p.Gly826_Ala828delinsAspThr	MCT	0.5[b]	Activated[b]	NA	NA
Cats	Exon 6	p.Met319_Thr322delinsIle	MCT	5.9[p]	NA	NA	NA
	Exon 8	p.Glu415Lys	MCT	4.2[s]	NA	NA	NA
		p.Glu415_Thr418dup{Glu415Gln}	MCT	40.3[p], 8.3[s]	Activated[p]	Suppression[p]	CR/PR (6)[p,t]
		p.His419_Ser421delinsLeu	MCT	4.2[s]	NA	NA	NA
		p.His419_Leu422delinsLeuIle	MCT	1.6[p]	Activated[p]	Suppression[p]	NA
		p.His419_Ser421delinsLeu	MCT	3.2[p]	Activated[p]	Suppression[p]	NA
	Exon 9	p.Ser477Ile	MCT	19.4[p], 41.7 [s]	Activated[p]	Suppression[p]	PR (2)[p]
		p.Glu491_Asn497del	MCT	4.2[s]	NA	NA	NA
		p.Arg493Lys	MCT	4.2[s]	NA	NA	NA
		p.Asn506Ile	MCT	4.8[p]	Activated[p]	Suppression[p]	PR (1)[p]
	Exon 11	p.Tyr554_Glu555delinsCysLysThrGln	MCT	1.6[p]	NA	NA	NA
		p.Glu555K	MCT	4.2[s]	NA	NA	NA
		p.Trp558_Lys559del	GIST	NA[u]	NA	NA	NA
		p.Glu563Lys	MCT	4.2[s]	NA	NA	NA

NA, not available; MCT, mast cell tumour; GIST, gastrointestinal stromal tumour; MEL, melanoma; AML, acute myeloid leukaemia; CR, complete response; PR, partial response. [a] Takeuchi et al. (2013); [b] Letard et al. (2008); [c] Kobayashi et al. (2012); [d] Yamada et al. (2011); [e] Nakano et al. (2014); [f] Ma et al. (1999); [g] Gregory-Bryson et al. (2010); [h] 1/4 dogs had a mutation, Frost et al. (2003); [i] Kobayashi et al. (2013); [j] Liao et al. (2002); [k] Chu et al. (2013); [l] Usher et al. (2009); [m] Webster et al. (2006); [n] Zemke et al. (2002); [o] London et al. (1999); [p] Isotani et al. (2010); [q] Isotani et al. (2008); [r] Our laboratory unpublished data; [s] Sabattini et al. (2013); [t] Isotani et al. (2006); [u] Morini et al. (2011); [v] Mutations K^{557} InsF and K^{557} N InsP in the original report are identical with the mutation p.Lys557delInsAsnPro (confirmed with the author of the original report).

type of canine *KIT* mutation, the exon 11 ITD mutation, shows variation in location and in duplicated size, but these ITD mutations commonly alter the gene sequence around codons 570–590 and have been shown to cause constitutive activation of KIT. Therefore, despite the observed variation, the exon 11 ITD mutations are likely to act as activating mutations.

Eight types of mutant KIT due to an activating mutation, (dogs: p.Glu417_Thr420dup{Glu417Gln}, p.Asn508Ile and ITD around codon 570–590; cats: p.Glu415_Thr418dup{Glu415Gln}, p.His419_Leu422delinsLeuIle, p.His419_Ser421delinsLeu, p.Ser477Ile, and p.Asn506Ile) have been demonstrated to be sensitive to imatinib in vitro. Moreover, tumours with these imatinib-sensitive mutations, except for two types of exon 8 mutations in cats, showed objective responses to imatinib in the clinic. Therefore, these activating mutations are likely to be imatinib-sensitive driver mutations and may be useful biomarkers for individualisation of imatinib therapy.

The activation status and susceptibility to imatinib in vitro of canine KIT with the mutations p.Gln555_Lys557del, p.Trp556_Val558delinsPhe and p.Val559Asp have not been examined; however, tumours with these mutations clearly responded to imatinib in the clinic. Although further molecular analyses are needed, these mutations may also be useful for individualisation of imatinib therapy.

Although seven types of KIT mutations (p.Ser479Ile, p.Gln555_Lys557delinsVal, p.Trp556Arg, p.Trp556_Lys557del, p.Val558del, p.Leu575Pro and p.Gly826_Ala828delinsAspThr) in dogs have been shown to be activating mutations, it is not known if the resulting mutant KITs are sensitive to imatinib in vitro and in the clinic. A KIT with an activating mutation is not always sensitive to imatinib. Sensitivity of a KIT mutant to imatinib is variable and depends on the location of the mutation and/or the property of the amino acid mutated. For example, in human mastocytosis, the vast majority of adult patients possess an activating *KIT* mutation in exon 17 that results in the mutation Asp816Val in the activation loop of KIT (Garcia-Montero et al., 2006). However, tumours carrying this mutation have been shown to be resistant to imatinib (Pardanani, 2013). Likewise, although imatinib has activity against human GISTs with a mutation in *KIT* exon 9, this activity is inferior when compared with GISTs with mutations in other *KIT* exons (e.g., exon 11) (Heinrich et al., 2008). Therefore, molecular and clinical analyses regarding the response of differently mutated KITs to imatinib are needed to clarify their value as targets of imatinib.

Almost half of the *KIT* mutations identified in canine and feline tumours have not yet been characterised with regard to activation status and imatinib susceptibility. However, detection of specific mutations in *KIT*, such as exon 11 ITD mutations in canine MCTs and exon 8 ITD mutations in feline MCTs, is likely to be valuable for individualisation of imatinib treatment. Further studies evaluating the relationship between *KIT* mutation status and the therapeutic effects of imatinib in canine and feline malignancies are necessary to enable the individualisation of imatinib therapy.

Conclusions

Imatinib has therapeutic activity towards a certain subset of canine and feline MCTs. In addition to MCTs, other tumours such as canine GISTs could respond to imatinib therapy; further clinical studies are needed to verify imatinib efficacy towards these tumours. Since the therapeutic activity of imatinib is associated with the presence of constitutively activated KIT, it would be beneficial to individualise imatinib treatment according to the mutation status of *KIT*. Some *KIT* mutations, especially those such as exon 11 ITD mutations in dogs and exon 8 ITD mutations in cats, are promising candidate biomarkers for individualisation of imatinib therapy for MCTs. Although imatinib appears to be safe for use in dogs and cats, further accumulation of cases and long-term follow-up are needed before making conclusions regarding the safety and toxicity of imatinib.

Conflict of interest statement

The author of this paper has no financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of this paper.

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